PD-1 blockade activates conventional CD4+ T cells and the innate immune response during glioblastoma eradication

Sarah Klein1, Jennifer Ziello2, Maria Speranza1, Praphulla Gokhale3,4,5, Paul Kirschmeier4,5, Katherine Crosby3, Gordon Freeman2,3, and David Reardon4,6

1Cell Signaling Technology, Inc., 2Dana-Farber Cancer Institute, 3Harvard Medical School, 4Belfer Institute for Applied Cancer Science, 5Lurie Family Imaging Center, 6Brigham and Women’s Hospital

ABSTRACT

Blockade of immune cell co-inhibitory receptor PD-1 using monoclonal antibodies enables anti-tumor immune responses in various solid tumors and lymphomas malignancies. On laboratory previously demonstrated PD-1 blockade effects on anti-tumor immune responses, but tumor necrosis and long-term survival in approximately 25-50% of mice with orthotopic GBM xenografts. Here, we evaluated the role of conventional CD4+ T cells and the innate immune response in PD-1 mediated antitumor immunity using multiple toxicology and flow cytometry. In response to PD-1 monotherapy immunotherapy, conventional CD4+ T cells express significantly elevated levels of proteins required for T cell proliferation, activation, and effector function. CD4+ T cell activation was accompanied by the classical activation and M1 polarization of resident macrophages and tumor infiltrating macrophages, including down-regulation of PD-L1 and upregulation of MHC class II surface expression. We also demonstrated that depletion of non-conventional CD8+ T cells was sufficient to completely ablate PD-1 mediated tumor necrosis and long-term survival. Our data implicates CD4+ T cells and macrophages play a key role in the eradication of glioblastomas by PD-1 blockade.

BACKGROUND

• Combination PD-1 and CTLA-4 antibody blockade induces tumor necrosis, and long-term survival in approximately 75% of mice bearing advanced stage glioblastomas in the GBM222 Highlight.

• Intratumoral accumulation of CD8+ cytotoxic T cells was observed in tumors treated with both anti-PD-1 and anti-CTLA-4.

• PD-1 monotherapy induced tumor necrosis in 25-50% of CD8+ glioblastoma-bearing mice, despite a decrease in CD8+ cytotoxic T cells.

• PD-1 or single agent treatment resulted in fewer CD4+ regulatory cells and myeloid-derived suppressor cells, as shown enhanced NK cell activation.

METHODS

C57BL/6 mice (The Jackson Laboratory) were orthotopically implanted with GBM222 cells 7 days post mating. Glioblastoma blunt was inoculated into the left parietal cortex of 7-9-week-old mice. Treatment was started on day 10. Mice were euthanized on day 21. The tumor burden was assessed by number of tumors and number of tumor tissue samples. The results were analyzed using the Student’s t-test.

CONCLUSIONS

• CD4+ and CD8+ T cells, but not NK cells, are required for the therapeutic benefit of PD-1 blockade in GBM222 tumors.

• PD-1 blockade increased conventional CD4+ T cell accumulation in tumors.

• PD-1 therapy increased CD4+ T cell infiltration. Glioblastoma CD4+ T cells can be detected in PD-1 -treated tumors.

• PD-1 -treated tumors had lower PD-1+ and TIM-3+ immune cells, and elevated PD-1T- cells, suggesting a shift toward activation versus exhaustion.

• PD-1 therapy enhanced the classical activation of resident and tumor-infiltrating myeloid populations.

REFERENCES